THE

EMBRYOLOGY OF STOMOTOCA APICATA

AND

THE EMBRYOLOGY OF TURRITOPSIS NUTRICULA.

BY

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PART I.

THE

EMBRYOLOGY OF STOMOTOCA APICATA.

THE EMBRYOLOGY OF STOMOTOCA APICATA.

INTRODUCTION.

The material for this research was secured, and the observations on the living forms were made, during the summers of 1903 and 1904 while I was occupying a table at the United States Fisheries Laboratory at Beaufort, North Carolina. Stomotoca is not very abundant in the harbor at Beaufort. I found it there as early as the middle of June. It is most plentiful during July and early in August. A few specimens may also be taken until early in September. The eggs were obtained from medusae captured between July 10 and August 5. The adult animals could not be secured in large numbers; and, owing to the fact that each female lays only a few eggs the material for embryological study was limited. Therefore the greater part of the work the results of which are embodied in tis paper was done with living material. All the drawings, with the exception of those of sections were made from camera sketches of the living

forms. Blastulae and planulae ranging in age from five to twenty-seven hours were preserved and sectioned for the study of the various stages in the formation of the en~~t~~doderm and the other features of development which make their appearance during this period.

I wish to acknowledge my obligations to the Honorable George M. Bowers, Commissioner of Fisheries for the priveleges afforded me at the Fisheries Laboratory; and also to thank Dr. Caswell Grave, Director of the Laboratory for help and suggestions. The work was finished in the Biological Laboratory of the Johns Hopkins University. For the interest shown and for kind suggestions offered during my work I am very grateful to Professor W. K. Brooks.

DEHISCENCE.

The eggs are discharged at about five o'clock in the morning. The ectodermal epithelium of the ovaries becomes ruptured, in fact broken down; and by the movements due to the muscular contractions of the manubrium the eggs are set free into the cavity of the sub-umbrella. Then by the rhythmic contractions of the bell they are forced out of the bell cavity into the water outside. While the eggs are being

laid the medusa remains at one spot, unless disturbed, and keeps up a continuous and rhythmic contraction and expansion of the bell and proboscis. Thus as the eggs are liberated, one, two, or three at a time, they are almost immediately passed out with the ejection of the water from the bell cavity. This process of dehiscence lasts for a few minutes during which the medusa remains at the bottom of the aquarium. All the mature eggs are discharged without intermission in the process, unless the medusa is disturbed. In that case it frequently swims to another part of the aquarium and in a short time commences to discharge the eggs again. The eggs in the ovaries of *Stomotoca apicata* are usually all deposited at one time. Occasionally a few immature ones are left in the ovaries after the process of dehiscence. Whether these mature and are laid at a later time, or whether they are reabsorbed I am not able to decide.

As stated above, the eggs are laid at about five A. M. On several occasions I observed the process of dehiscence and found that the time was always practically the same. Some medusae were watched all night, July 14. At five o'clock in the morning they began to lay their eggs. They all began

at about the same time and all the eggs were discharged within fifteen or twenty minutes. The time when the medusae are captured and put into the aquarium does not seem to have any influence on the period of dehiscence. I have taken them in the tow at nearly all hours of day and night, and never have had them to deposit their eggs except at 5 o'clock in the morning.

THE EGG.

The egg of *Stomotoca apicata* is spherical and measures .14 of a millimeter in diameter. It is devoid of a membrane and the cytoplasm is rather dense and only semi-transparent; however it is not as dense as the egg of *Stomotoca rugosa*, which is extremely opaque and of a chalky-white color, and also slightly larger. The color of the egg of *Stomotoca apicata* is a bluish-white.

A point of interest may be mentioned in this connection. On one occasion, having taken a number of *Stomotoca* in the tow at night, they were picked out and put into a dish of clean sea-water with the intention of allowing them to lay, and using the eggs for study the next morning. It happened that both species of *Stomotoca* that are found at Beaufort

were represented. There were mature females of both species that deposited their eggs the next morning at the regular period; Stomotoca rugosa has the same time for dehiscence as Stomotoca apicata. Only the eggs of the latter species developed; there being no males of Stomotoca rugosa. The next day when the two species were in the same dish, and both discharged their eggs, only the eggs of Stomotoca rugosa segmented and developed. In this case there were no mature males of Stomotoca apicata. These facts aroused my interest and on several later occasions I placed the two species together with the intention of getting them to interbreed, but did not succeed and therefore I am led to the conclusion that they will not cross even though they are species of the same genus. To my knowledge no other experiments have been made in attempting to cross different species of this group of animals, and I did not have the opportunity to try with any other species than the above named after my attention had been called to the fact that they did not cross when accidentaly placed in a dish together.

POLAR BODIES.

Soon after the egg is deposited the first polar body

is given off. A few minutes later the second polar body is formed. They remain near the egg for some time; frequently until after the second or third segmentation. The polar bodies are not held by a membrane, as the egg is devoid of such a structure; neither are there any protoplasmic connections visible with a magnification of 212 diameters. Yet for a time they seem to be held near the egg by some means of attraction. The first polar body may segment once or twice. Usually about the time of the second cleavage the polar bodies either disintegrate or pass out into the water and are lost.

FERTILIZATION.

Very little concerning fertilization could be made out on account of the character of the egg. The ova and spermatazoa are discharged into the water and there fertilization takes place. It is impossible to follow the nuclear changes which take place during maturation; or the union of the male and female pronuclei in the living egg because of the density of the cytoplasm, and because material could not be secured in sufficient abundance in the various phases for the preservation

of the different stages for sections. There is no visible fertilization-membrane given off after the penetration of the spermatozoa.

CLEAVAGE.

Cleavage is total, equal and nearly regular, especially in the early stages. The divisions occur at short intervals, and the blastomeres soon move away from the center of the egg, thus forming a gradually enlarging segmentation cavity. The cells continue to divide and arrange themselves into a single layer around the blastocoele to form a true blastula. The egg is not divided into an animal and a vegetative pole as the deuteroplasm and protoplasm are distributed evenly in all parts. But as is customary and for convenience of description I will call the part of the ovum from which the polar bodies are given off the upper pole, and the part of the egg op osite the lower pole.

The first cleavage occurs a short time after the polar bodies are ejected. The plane of division is vertical; the segmentation-furrow begins at the upper pole and gradually deepens until the egg is cut into two equal parts. The egg,

viewed from above, at first shows a nearly circular depression which very soon spreads laterally and begins to grow down. This first furrow is wide and leaves the blastomeres separated some distance from each other as it progresses downward, as is seen by looking at the egg from the side (Figs. 4 and 5). This furrow remains open until the egg is almost separated into two parts; the blastomeres being connected simply by a narrow protoplasmic film at the lower pole. Protoplasmic currents can frequently be seen in this connecting thread. Bunting ('93) describes and figures in Hydractinia a protoplasmic thread in the two cell stage in which she also notes protoplasmic movements. The connecting film in Stomotoca apicata is not as clear and definite in outline as she shows it in her figure of Hydractinia. The two cells gradually come in close proximity and in a short time the connection of protoplasm at the lower pole is broken and the complete two-celled stage is formed (Fig. 6).

The second plane of division id also meridional and at right angles to the first. This cleavage takes place about fifteen minutes after the first division. These second segmentation furrows start at the centre and move out toward/the periphery. During their progress outward there are to be seen globular or oval spaces at their outer extremities. These spaces are large enough to cause openings that extend through the egg as shown in Figure 7. During this cleavage there is a shifting or rotation of the blastomeres from right to left. The second segmentaion furrows usually start opposite each other at a point in the centre of the first cleavage furrow, and then are carried apart by the rotation. Or the rotation may have started before the second segmentation began; in that case the second cleavage planes are some distance apart as soon as they make their appearance. Figure 7 shows an egg in the process of division in which rotation has taken place. During the progress of the second segmentation, the egg has fre-

quently a flattened appearance as seen in the figure just mentioned.

In this stage protoplasmic films or bridges, also, frequently exist for a time after the segmentation is practically complete. They finally are absorbed by the blastomeres which round up forming the completed four-celled stage as shown in Figure 8.

The third cleavage plane is equatorial and divides the egg into eight equal blastomeres; four of which are situated at the upper pole and four at the lower pole of the egg as seen in Figure 9. This is the condition when the ~~condition~~ segmentation is regular, and might be described as two four-celled stages of half size superimposed one upon the other, and then the upper set rotated to the left. While the formation of the eight-celled stage was always nearly the same in the eggs that I followed, after the division was completed, the blastomeres did not always retain the same relative positions. Sometimes there occured a separation of the cells at one side of the equatorial furrow and the blastomeres rolled

apart in such a manner as to form a curved sheet. In others this separation and unrolling of the blastomeres was less definite and the final arrangement was such as shown in Figure 10.

The irregularity in the relative position of the blastomeres begins with the eight celled stage and is more or less characteristic of all later stages up to the formation of the blastula. But, while there is diversity of arrangement of the blastomeres, nevertheless I am led to believe that the division of the individual cells is regular and takes place just as though the blastomeres always held the same relative position.

The fourth segmentation follows after a short period of time. Figure 11 shows a sixteen-celled stage which is nearly regular, but the cleavage cavity has already been formed within the mass of blastomeres and they are thus pushed away from the centre of the egg. In this stage the cell lineage can still be traced even in the forms that are somewhat irregular. But in the older stages the arrangement of

the cells is more irregular and owing to the opacity of the egg it is difficult to follow with accuracy the descent of the cells. Figure 12 shows a later stage in which the arrangement of the cells is more regular than is frequently met with in eggs of the same age.

As stated before, the divisions follow each other at short intervals. Within two hours after the eggs were laid they had undergone the process of maturation and fertilization, and had passed beyond the sixty-four celled stage. The cells continue to divide with the same rapidity, while within them the cleavage cavity is also gradually enlarging. Figure 13 shows a stage in which the cells are more or less definitely placed around the segmentation cavity. The blastomeres finally become very numerous and small, and arrange themselves around the blastocoele in a single celled layer forming a true blastula.

BLASTULA.

The blastula is oval in shape, and is but slightly

larger than the unsegmented egg. The average size of several blastulae that were measured was .19 mm. in length and .15 mm. in their largest transverse diameter. The egg before cleavage measured, as stated before, .14 mm. in diameter. The blastomeres in the blastula stage have become very numerous and small, and are arranged in a single layer of epithelial cells. When the larva is about eight or ten hours old, these peripheral cells develop cilia; probably each cell has one cilium. With the development of the cilia movement commences. At first the motion is slight, but as the cilia become more numerous, the blastula is enabled by the ciliary movements to leave the bottom of the aquarium upon which it was heretofore lying and to swim about in the water with a spiral or cork-screw motion which is characteristic of hydroid blastulae and planulae. The large end of the blastula is directed forward and therefore may be called the anterior end. Whether the anterior part of the larva corresponds to the upper or lower pole of the egg was

impossible to determine. It is reasonable, however, to infer that there may be no fixed polarity in the larva of Hydromedusae, for it is well known that normal embryos of small size will develope from fragments of eggs.

PLANULA.

The blastula gradually elongates and becomes narrower forming a larva which is usually about three times as long as broad and known as a planula. From measurements taken of living planulae the average size is about .25 mm. in length and .08 mm. in the short diameter. These measurements are not constant, the larva becoming somewhat longer at an older age. The anterior end remains slightly larger than the posterior, but the difference is not as great as in the blastula. During the blastula stage the larva swam near the bottom of the dish; when it attains the planula stage it rises and swims at or near the surface of the water for a shorter or longer time. This phenomenon occurs about twenty-four hours after the eggs are fertilized.

After several hours the planula gradually settles toward the bottom again and finally the spiral movements cease, due to the loss of the cilia. For a time of varying length after the spiral motion stops the planula glides along on the bottom of the aquarium. About forty-eight hours after the eggs are laid the larva reaches the stage of development in which attachment takes place. In preparation for attachment the planula settles to the bottom, loses its cilia and ceases its movements.

FORMATION OF THE ECTODERM.

The formation of the ectoderm in *Stomotoca apicata* is simple in comparison with that of those species in which the segmentation of the egg is unequal, giving rise to macromeres and micromeres; and in which the ectoderm is formed by a rapid increase of the micromeres and overgrowing of the macromeres by the process of epibole. In *Stomotoca* on the other hand the cleavage is equal and at the completion of segmentation the blastomeres have divided into cells of uni-

form size and are situated in a single epithelial layer around the periphery of the blastula (Figures 16 and 17 show sections of blastulae five and eight and one half hours old respectively). Thus, from their position, all the cells which result from the segmentation of the egg directly may properly be regarded as forming ectoderm; and indeed might already at this stage of development be designated as such, were it proper to use the term ectoderm before the appearance of an inner germ layer. The cells of the blastosphere are columnar in shape and at first all are comparatively of the same height; but finally those cells at the posterior end become somewhat taller than the rest. This is the region where the endoderm will be budded off.

FORMATION OF THE ENDODERM.

In Stomotoca the formation of the endoderm takes place by unipolar ingression, or the "hypotrope" method. The latter term was used by Metschnikoff in contradistinction

to multipolar migration. In the multipolar formation of the endoderm he distinguishes four different modes, namely: 1. A primary delamination which takes place by a transverse division of the blastoderm cells, and occurs in the Geryonidae and *Eudendrium*. 2. A multipolar ingression which takes place on all sides (Aeginopsis). 3. A secondary delamination which occurs where a morula structure exists, as in *Aglaura*, *Rhopalonema* and in most of the hydroid polyps. 4. A mixed delamination in which the endodermal cells originate in part through transverse division or ingression; and, also, through subsequent differentiation as a secondary delamination. This last mode of the formation of the endoderm, according to Metschnikoff, occurs in *Polyxenia*; and is the transitional method between multipolar migration and epibole. In the unipolar ingression, or "hypotrope" process the formation of the endoderm is confined to a comparatively small area at the posterior end of the blastula. This is the method that is followed in the species under

consideration.

About the time the blastula becomes ciliated and begins to swim, usually eight to ten hours after fertilization, the cells at the posterior end of the larva become somewhat taller than those in the other regions; and from these cells relatively few in number, the endoderm arises. The formation of the endoderm in Stomotoca is, in a general way, similar to that described by Metschnikoff in his "Embryologische Studien an Medusen" for Clytia flavidula, Clytia viridicans and Octorchis Gegenbauri. The endodermal cells are given off from the lower end of the blastula and are pushed into the blastocoele. At first a single cell may be budded off. Gradually more cells are given off, and those first set free divide; so that by the continuation of this process for an indefinite time, the blastocoele becomes filled solidly from the anterior to the posterior end. Figures 18, 19 and 20 are from sections of blastulae in which the formation of the endoderm is in different

stages of progress; and in Figure 21 the endodermal tissue has filled the entire cavity.

According to Metschnikoff, in his description of unipolar ingression or "hypotrope," the endodermal tissue arises as a rule by bodily migration of endodermal cells into the blastocoele, and not by a transverse division of the ectodermal cells-- the inner parts going to form endoderm and the outer parts remaining as ectodermal cells. In Figure 20, Plate 2 Metschnikoff shows a cell in the process of transverse division; and in Figure 21 of the same Plate two cells are so situated that one can easily infer that they may have arisen by transverse division of a single ectodermal cell. These figures are of *Clytia* and in his description of the same species he mentions the cell in Figure 20 as the only one that he found in which transverse division occured. This he seems to regard as an exception, and claims that as a rule the ectodermal cells increase by longitudinal division and migrate into the interior.

My material for studying the formation of the endoderm in *Stomotoca* was scarce and it is not impossible to have misinterpreted the phenomena. However, I am inclined to think that the endodermal cells arise by a transverse division of the ectodermal cells, as Metschnikoff shows in the exceptional case of *Clytia viridicans*. Figure 18 is drawn from the only section I was able to secure from preserved material showing the beginning of the formation of the endoderm, and that section was cut slightly oblique, causing some doubt. A section of a little older stage and drawn with higher magnification is shown in Figure 19. Here there are three cells that appear to have just divided by transverse division. Another reason which causes me to think that the endodermal cells arise by transverse division of the original ectoderm cells is the fact that the ectodermal cells in this region are practically as wide as those in other parts of the blastula. This would not be the case if the longitudinal division occured; for necessarily cell division

is more rapid in the region where the endoderm is given off, and consequently the cells would be narrower. Unfortunately, because of scarcity of material, the exact cellular details of the formation of the endoderm will have to be left for future study.

The migration of the endoderm continues for some hours, and finally the blastocoele becomes solidly filled with this newly developed tissue. At first the cells are crowded together, frequently quite densely, without any definite arrangement except that due to pressure. Then those cells that are situated next to the ectodermal layer change in shape, become columnar and assume the appearance of a more or less distinct layer. Such an arrangement is shown in Figure 22. Later a separation takes place in the centre of the endodermal mass. This is the first beginning of the coelenteric cavity, which gradually increases in size; and finally the endodermal cells become arranged in a single layer around this cavity.

DIFFERENTIATION OF THE ECTODERMAL CELLS.

When the larva is about twenty-four hours old and about the same time that the endodermal tissue begins to arrange itself into the definite inner germ layer, a differentiation commences in the ectodermal tissue. The interstitial cells now make their appearance here and there by crowding in between the bases of the ectodermal cells. These latter cells which heretofore were straight cylindrical structures with their sides parallel to each other, now become more irregular; some assume conical forms, others spindle shapes according to the pressure of the neighboring cells. Also, about this time, or a little later, small oval refractive bodies make their appearance usually in the interstitial cells, occasionally in the ectodermal cells also. These small ovoid structures gradually push their way toward the exterior, and finally come to be situated in or between the ectodermal cells at the surface. They are developed into nematocysts.

ATTACHMENT.

When the larva is about forty-eight to fifty hours old it settles to the bottom, loses its cilia and thus its movements cease. It is now ready to become attached. The method of attachment in *Stomotoca* differs from that usually described and ~~which is~~ regarded as typical for the hydroid larva; in which case they settle down on the broad anterior end, from which the hydrorhiza are given off, while the opposite end forms the hydranth and develops the mouth and tentacles. The planula of *Stomotoca* instead of settling down on the anterior end, becomes attached by the whole length of the larva. That is, the planula does not become transformed into a hydranth but forms the root; and the first hydranth is given off from the root as a bud. The planula changes its shape about the time it is ready for attachment. The enlarged anterior end is reduced in size and the larva becomes spindle shaped. Then usually about the time the bud which will form the hydranth appears, the

primary root branches, giving off one or two secondary roots; so that when the hydranth is developed it may have two, three or four hydrorhizae, as shown in Figures 27 - 29. The settling down and attachment of the planula of _Stomotoca apicata_ is very much like that which takes place in _Turritopsis nutricula_, the development of which will be described in another paper.

Professor Brooks in his work on "The Life-History of Eutima" (1884) has shown that the planulae of _Eutima_, _Turritopsis_ and _Hydractinia_ form roots and that the hydranths arise as buds from the roots.

DEVELOPMENT OF THE HYDRANTH.

After the larva has become attached it very soon develops a bud, generally at about the centre of the root, which is the beginning of the hydranth. A circle of small projections make their appearance very early around the distal end of the hydranth bud; these are the rudiments of the tentacles and are usually five in number. Occasionally

a hydranth bud is met with which has six tentacular pro- jections and thus gives rise to six primary tentacles. The mouth is now developed, as a slit breaking through the two germ layers, at the apex of the young hydranth in the centre of the whorl of tentacular buds. About a day later more tentacles appear. These secondary tentacles alternate with the primary ones. The secondary tentacular buds do not all appear simultaneously; but are usually added one or two at a time until the second cycle of tentacles is completed and the hydranth has ten tentacles in all. Thus we may have young hydranths with six, seven, eight, nine or ten tentacles according to the stage of development. Ten seems to be the number of tentacles in the fully developed hydroid polyp. The oldest polyps that I reared five days old had this number; and Professor Brooks described the hydroid, which he found on the lower surface of the shell of the living limulus, and which had medusa buds, developed, as having only ten tentacles. The hydranths

that I reared in the laboratory correspond with those found by Professor Brooks and I have no doubt that they are the same species. The primary and secondary tentacles arise from the same level so that they may be said to constitute one whorl. The five primary tentacles, however, are longer and project forward; while the secondary ones are shorter and extend backward. The tentacles are well armed with thread cells which are arranged around the tentacles in clusters at short distances from each other, from one end of the tentacle to the other. These groups of thread cells become closer together as the distal end of the tentacle is approached.

A thin delicate perisarc is secreted early in the development of the hydranth. It adheres closely to the root and stem. It does not extend the entire length of the stem; but stops a little distance below the circle of tentacles. In Figure 31 a polyp is shown in which the coenosarc has retracted for some distance in one of the hydrorhiza and

left the delicate tube of perisarc empty.

SUMMARY.

1. The eggs are laid at a regular time, about five o'clock in the morning. They are set free by the breaking down of the epithelial layer of the ovaries.

2. The egg is spherical and measures .14 mm. in diameter. It is destitute of a membrane when laid, and none is subsequently developed. The cytoplasm is dense and opaque.

3. Maturation takes place after the eggs are laid; and fertilization takes place very soon. Details of fertilization could not be made out because of opacity of eggs.

4. Cleavage is total, equal and nearly regular, especially in the early stages. Protoplasmic threads or bridges, connecting the different blastomeres during the early cleavages, are frequently encountered. The segmenting cells arrange themselves around a continually enlarging cleavage cavity.

5. At the completion of the segmentation a true blastula is formed, which develops cilia and swims with a spiral motion. The oval blastula elongates and is transformed into planula.

6. The ectoderm arises directly from the segmentation cells which are arranged in a peripheral layer around the blastocoele.

7. The formation of the endoderm is by unipolar ingression. The cells at the posterior end of the blastula bud off the primitive endoderm tissue which migrates into the blastocoele; and later is arranged into the inner germ layer.

8. Nematocysts arise chiefly in the interstitial cells, sometimes in the endoderm, and migrate to the surface.

9. The larva becomes attached by its side and is transformed into the hydrorhiza. The root frequently branches soon after attachment.

10. The hydranth develops from a bud, which is given off from about the centre of the hydrorhiza.

11. The tentacles appear early as small projections at the distal part of the hydranth bud.

12. A thin delicate perisarc is secreted around the hydrorhiza and extends up to near the tentacles.

PART II.

THE

EMBRYOLOGY OF TURRITOPSIS NUTRICULA.

THE EMBRYOLOGY OF TURRITOPSIS NUTRICULA.

INTRODUCTION.

This work on the embryology of *Turritopsis nutricula* was begun at the suggestion of Professor Brooks. The material was collected and the observations on the living specimens were made during the summers of 1903 and 1904, while I occupied a table at the United States Fisheries Laboratory at Beaufort, North Carolina. Turritopsis is one of the most common medusae in the harbor during the summer. In the two years that I was there they became abundant in the beginning of July and remained more or less plentiful until I left Beaufort September 13. While the medusae could be collected in fairly large numbers, many of them were immature; they lay only a limited number of eggs. However the material was preserved and sectioned for the study of such facts as could not be made out from the living forms. The work was finished in the Biological Laboratory of the Johns Hopkins

University.

DEVELOPMENT OF THE OVARIAN EGG.

The ova develop in the ectodermal layer of the manubrium. The epithelium becomes very much thickened in four regions; these enlarged areas form the ovaries. The primitive ovarian cells when first differentiated are larger than the ectodermal cells of other parts. Their protoplasm becomes homogeneous and of a finely granular character. The nuclei are less hyaline in appearance; and the nucleolus stains deeply. The primitive ova are first distinguished from the rest of the ovarian cells by the increase in the density of the cytoplasm and the enlarging of the nucleus. The latter becomes very large in proportion to the size of the cell; and acquires a vescicular character. The nucleolus is conspicuous, and a network of chromatin is scattered through the germinal vesicle.

The primitive ova grow by the absorption of the ovarian cells around them. As growth takes place there is a change

in the character of the cytoplasm. It loses its homogeneous and finely granular nature and develops a supply of deuteroplasm in the form of yolk granules. These are large and stain very darkly. They first appear around the germinal vesicle. As they become more numerous by the continual formation of new ones, they are pushed out through the cytoplasm toward the periphery. The formation of the yolk spheres goes on until the ovum is densely crowded with them except for a narrow peripheral zone, in which the protoplasm retains its homogeneous and finely granular character and forms the ectoplasm of the mature egg. Figures 1 to 6 inclusive show different stages in the development of the ovarian egg and the formation and migration of the yolk granules. Some idea of the extent to which the protoplasm becomes crowded with spheres of deuteroplasm can be formed from Figure 6, which is drawn from a nearly mature ovum. In the fully developed egg the layer of ectoplasm is narrower than is represented in this figure.

The yolk granules first appear around the nucleus of the

ovum; and it is not improbable that they are, in part at least, the result of nuclear activity. During the formation of these bodies, the nucleolus shows signs of being in an active condition and it may also be connected with their manufacture. In some stages the nucleolus is dense and homogeneous; in others it has one or two clearer globules in its interior. These facts seem to show that it is not in a dormant state; and it is possibe that it may be associated in some way with the transformation of the absorbed protoplasm into deuteroplasm; at least that the yolk spheres arise directly through the activity of the cytoplasm, independently of any nuclear or nucleolar function, is doubtful. ~~For~~ If ~~such~~ this were the case we would expect the yolk bodies to arise in other parts of the ovum than around the germinal vesicle. That this occurs there is no evidence from the study of many eggs. The primitive ovarian cells are all, or nearly all, absorbed and used in the manufacture of the yolk granules by the growing ova, except a layer at the outside which is transformed into the epithelium of the ovary. The cells

of the ovarian wall are small and somewhat flattened. Their nuclei are about the same size as the nuclei of the primitive germ cells, but are less dense. The nucleoli are conspicuous and stain deeply. In general the cells of the epithelium of the ovary are similar, except they are not as much flattened, to the cells in other parts of the ectodermal layer of the subumbrella. The eggs in the ovary lie next to the mesogloea, that is, there is no ectodermal tissue between them and the supporting layer. The ovarian eggs are irregular in shape due to their being crowded together; but when liberated they become spherical.

DEHISCENCE.

The eggs are imbedded in the ectodermal layer of the manubrium. As the ova grow and increase in size the epithelium of the ovary becomes more and more distended. When they have reached maturity the outer ectodermal tissue of the ovary is under considerable tension. Finally when the time for dehiscence arrives, the outer wall of the ovary is ruptured by the aid of the muscular contractions of the manubrium

and bell and the eggs escape into the acvity of the umbrella. The process of egg laying is very similar to that described for Stomotoca.

The number of eggs deposited by a single female medusa varies considerably. It is usually between twenty and thirty five. On one occasion an exceptionally large female was taken in the tow; her ovaries were seen to be crowded with eggs. She was put into a separate dish of sea water for the purpose of counting the number of eggs that she would lay. The next morning at the regular hour the eggs were deposited; and the number was found to be fifty-six, which is unusually large. I made many other counts but this was the only time that the number exceeded fifty. As a rule it is from twenty to thirty-five, only rarely is it as high as fifty. These numbers seem remarkably small when we consider the enormous quantities of eggs that are laid by many of the other animals of the ocean; the number often reaching many millions, as among some of the Echinodermata and Mollusca.

It is a rather curious fact that these animals are

always so very regular in the time for depositing their eggs, which is from five to six A. M. During the two summers that I studied *Turritopsis* at the sea-shore, great numbers were collected and kept in aquaria. On many occasions I arose early in the morning to observe the act of spawning,- one time they were watched through the entire night,- and always the act of egg laying was seen to commence at about five o'clock or a few minutes after. Very rarely did it take place as late as six o'clock; and on no occasion was the phenomenon observed more than a few minutes before 5 A. M.

This precise periodicity is not ~~only~~ confined to *Turritopsis*, but seems to be quite prevalent among the medusae in general. In *Stomotoca apicata*, *Stomotoca rugosa* and a species of *Eucheilota* I find that the eggs are deposited also at a fixed hour, namely, 5 to 5.30 A. M. Professor Brooks found that *Lirope* and *Eutima* spawn at about 8 P. M. In *Gonionema* Perkins found the time to be from 7 to 8 P. M. Bunting found the period of dehiscence for *Hydractinia* to be about 10 P. M. While Merejkowsky says that the eggs of

Obelia are laid early in the morning. Metschnikoff also gives the time of spawning of 14 species.

Regular breeding habits have also been found to exist among other marine animals, and may be more general than has been suspected. Wilson in his work on the development of *Renilla* found that the eggs of that form were always laid at about 6 A. M. In a single case only, he says, the spawning took place as early as 5.30 and it was never observed to occur later than seven o'clock. The pelagic Crustacean, *Lucifer*, Professor Brooks observed to deposit its eggs at 9 to 10 P. M.

Bunting found that by packing *Hydractinia* in ice and keeping them at a lower temperature she was able to delay the time of egg laying. On restoring the animals to the normal temperature, the eggs were laid after a short period of time. Perkins found that the periodicity of spawning in *Gonionema* is definitely effected by changes of light. By placing his medusae in a dark place for an hour and then putting them in the daylight apparently normal egg laying again took place.

While I did not try experiments on Turritopsis either with regard to temperature or light, yet the changes of temperature from day to day had no noticeable effect on the time at which they discharged their eggs, that is, it occured at the same hour on warm days and cool days. In like manner the fact that the aquarium in which the medusae were contained was kept before a lighted lamp all night had no effect. on the time of spawning the next morning, which took place at the fixed period.

THE EGG.

The egg of Turritopsis is spherical and ~~is~~ devoid of a membrane when first laid and none is subsequently formed. In size it is quite small and can easliy be overlooked. If the water is free from sediment and the dish containing the eggs is placed upon a piece of black paper the eggs are visible to the naked eye. They measure .116 of a millimeter in diameter. They are among the smaller of the medusae eggs. Metschnikoff gives the measurements of the ova of nineteen

species of medusae; the sizes of which range from .024 mm. to 1.5 mm. *Cunina proboscidea* has the smallest and *Polyxenia albescens* the largest egg of the species included in his list. The egg of *Turritopsis* is just slightly larger than that of *Rathkea fasciculata* according to the measurement of Metschnikoff.

In the substance of the egg two parts are distinguishable; an outer layer of clearer ectoplasm which consists of viscid formative yolk composed of protoplasm with very fine granules; and a central mass of endoplasm which is dense and opaque and filled with large, dark granules of nutritive yolk. From the fact that the endoplasm is crowded with these coarse dense granules of nutritive material the egg is very opaque and the germinal vesicle is not to be seen from the exterior. Thus the changes which take place during maturation and fertilization, and the nuclear phenomena of segmentation, as well as the formation of the endoderm cannot be followed in the living egg. For this reason

the egg of Turritopsis is not as suitable for study during life as those beautifully transparent eggs of Liriope and Eutima for instance, which allow all the changes that take place within the egg during development to be followed easily.

The specific gravity of the eggs is greater than that of sea-water and consequently they sink to the bottom of the aquarium as soon as they are discharged from the cavity of the umbrella. In opacity the egg of Turritopsis is intermediate between the egg of Stomotoca rugosa, which is extremely dense and of a chalky white color, and the egg of Stomotoca apicata which is semi-transparent and appears bluish-white by reflected light. In color the egg of Turritopsis is yellowish white.

MATURATION AND FERTILIZATION.

Because of the opacity of the egg satisfactory observations on the phenomena of maturation and fertilization are impossible during life, except for those changes which

take place on the outside. A few minutes after the egg is laid the first polar body is given off at the upper pole of the egg. The second polar globule follows after a very short interval. These structures are of an ephemeral nature and soon disintegrate or pass out into the water and are lost. Nothing can be made out of their internal structure or ~~and~~ of the arrangement of the chromatin with the low magnification which one is obliged to use in the study of the living egg. However I was fortunate enough to get sections of the early stages of preserved eggs which show the polar bodies in the process of being extruded. The germinal vesicle moves to the periphery of the egg, then a part of its substance is divided off and extruded as the first polar body. In Figure 7, which is a section of an egg that was preserved a few minutes after it had been laid, the second polar body is just being given off. It contains several granules of chromatin scattered through its clear hyaline substance. [illegible] a little distance

from the egg, but is still held in connection with it by some means of attachment, the chromatin has come together and forms a single mass in the centre of the polar globule. The means of attachment of the polar bodies to the surface of the egg is not quite clear, as the egg is destitute of a membrane. It is possible that some of the clear liquid part of the protoplasm may exude from the substance of the egg as the polar bodies are extruded and be the means of holding them to the surface of the egg even during fixation.

As can be seen in the figure, the germinal vesicle during the extrusion of the polar bodies is situated at the very edge of the egg; even, about half of its bulk extends beyond the general contour of the egg's surface. The yolk granules are crowded around the nucleus with the same density as in other parts of the egg. After the second polar body has been given off, the female pronucleus moves back from the periphery some distance. Here it is met by the sperm nucleus and fusion of the two takes place. Whether there is

any definite spot for the entrance of the spermatozoon or not could not be decided. But I am inclined to think that the male element is capable of penetrating the egg at any part; and that when it has once entered the substance of the egg, the male and female pronuclei are brought together by the attraction existing between the two.

It was impossible to see the discharge of the spermatozoa from the males; neither did I see them enter the eggs. And, as stated before, the eggs are so opaque that the internal phenomena of fertilization could not be followed in the living specimens. ~~But~~ There is reason to believe that the sperms are discharged at about the same time that the females lay their eggs. Fertilization takes place in the water immediately following maturation, and segmentation begins in a very short time.

SEGMENTATION.

Segmentation is total and approximately equal. While there is a slight difference in the size of the blastomeres

at times, yet this difference is not constant and they all have the same value in development; that is, they are not divided in to macromeres and micromeres. ~~And~~ There is no evidence either from observations of the living eggs, or from the study of sections of preserved material that any of the blastomeres can be localized as forming distinct parts of the future embryo. During the first two or three cleavages the process is usually quite regular, but beyond the eight cell stage the segmentation becomes very irregular and erratic; almost if not fully as remarkable as that described and figured by Hargitt for Pennaria tiarella and of which he says: "Between the extremes of the embryonic history from the early cleavage to the formation of the morula are to be found the most erratic and anomalous exhibitions of developmental phenomena which have ever come to my knowledge, if indeed its counterpart has hitherto been known. It is not strange that with the mental pictures of such steady-going exhibitions as are found in the development of annelids, molluscs, etc., one should regard such

monstrosities as are very inadequately represented in the various figures illustrating this paper as abnormal to the degree of being pathalogic! And thus it seemed to me when first observed; and as pointed out in the earlier paper, the first batch of eggs were discarded as having 'gone bad.' "

When I first began the study of the development of Turritopsis, the irregularities of segmentation struck me as very peculiar and I was at first inclined to think that they were abnormal. After I allowed the eggs time to progress I discovered that they developed into normal planulae and thus was forced to conclude that this strange and irregular cleavage must after all be normal for the species. On several occasions the attention of a number of other observers who were working in the same marine laboratory was called to this phenomenon, and they also expressed surprise and remarked that they had never seen segmentation presenting such anomalous and irregular features.

Metschnikoff describes and gives a few figures of a very similar condition of segmentation in Oceania armata.

He says: "Wenn bei den beschriebenen Medusen verschiedene Abweichungen in dem Zustandekommen des vierten Furchungsstadium constatirt werden mussten, so konnte man doch bei allen eine gewisse Regelmässigkeit auffinden. Ganz abweichend in dieser Beziehung verhält sich *Oceania armata*, da bei dieser Meduse die kaum mit einander vereinigten Blastomeren durchaus unregelmässig und ordnungslos nebeneinander liegen. - - - Das Abweichende in der Embryonalentwickelung der *Oceania armata* hört noch nicht so bald auf. Die Furchung setzt sich in unregelmässigster Weise fort und führt zur Bildung unförmlicher Zellenhaufen, in deren Innern man eine Furchungshöhle durchschimmern sieht. Oft nehmen solche Embryonen eine ganz abenteurliche Gestalt an, deren Ursache zum Theil darin liegt, dass sie sich durch Theilung vermehren. Diesen Process habe ich an mehreren isolirten Blastula-Stadien beobachtet, so dass ich an dessen Existenz nicht zweifle." In *Turritopsis*, likewise, the later cleavages take place in a most irregular manner and lead to the for-

mation of a shapeless and grotesque mass of blastomeres in which the cells are frequently held together very loosely. The accompanying drawings unfortunately represent only the most regular forms. This is due in part to the fact that the very irregular forms were at first thought, as stated before, to be abnormal; and partly because it was difficult to make accurate camera sketches of these shapeless masses during life while cleavages were ~~skip~~ taking place rather rapidly.

Whether these embryos multiply by division, as Metschnikoff stated to be the case with Oceania armata and to which he attributed in part the cause of their peculiar shapes, I have no direct evidence; but think that it is very probable that such may be the case. ~~nor~~ Frequently the blastomeres are separated into two distinct masses held together by a small isthmus of cells; even if they do not divide by an internal activity, they must, occasionally at least, be broken apart by the action of the tides when in the open ocean. Several times the experiment of dividing the egg during the

comparatively early cleavages was tried and the parts were found to continue their development without any hindrance. These experiments will be described more in detail later.

Another point in which the segmenting egg of _Turritopsis_ differs from that of _Oceania armata_ is that it does not form a true cleavage cavity. The blastomeres always form a more or less solid embryo, as shown in the sections of these stages. Occasionally there are small spaces left between the cells; but a true segmentation cavity that later forms a blastocoele is never formed. In this respect also it is similar to the development of _Pennaria tiarella_ as described by Hargitt. As the completion of segmentation approaches, these irregular masses of cells gradually take on a more symmetrical form and finally there is formed an oval embryo composed of a solid mass of cells constituting a morula.

The first cleavage takes place about twenty to thirty minutes after the polar bodies have been given off. It begins at the upper pole of the egg and passed down to the

lower pole. Thus the egg is divided meridionally into two cells of approximately equal size. When the division is complete the blastomeres do not remain in close union, but move apart so that the two spheres are connected by only small arcs of their circumference. The protoplasmic bridge, which frequently occurs in hydroid eggs at the lower pole just previous to the completion of the two-celled stage, is usually to be seen in the egg of this species; but it is much less conspicuous than is the case in Stomotoca. And when it occurs is less definite and clearly defined than is the condition in Hydractinia, as described and figured by Bunting. Metschnikoff also figures a very beautiful example of this protoplasmic connection in the egg of Nausithoe marginata. In Turritopsis the condition is much like that of Rathkea fasciculata, as shown by the last mentioned observer, in which the connections instead of becoming a very definite bridge remain for a time as a less clearly outlined portion of the ectosarcal material. Protoplasmic

currents may be seen at times in these connecting filaments. Their function does not seem to be clearly known; but it, very probably, is connected with a readjustment of the cytoplasm and the establishment of an equilibrium between the different blastomeres.

Hargitt in his paper on "The Early Development of Pennaria tiarella" discusses the occurence of papillae, threads, and bridges; and reviews briefly the observations of a number of other investigators in regard to these phenomena, and the cytoplasmic activities which they have seen to take place in the eggs of a number of animals widely separated morphologically. No definite conclusions are reached as to the functions of these various phenomena, but it is generally thought that they are concerned with fundamental intrinsic changes within the cytoplasm.

These protoplasmic connections are usually composed of the ectosarc only. They are present not only in the two-celled stage, but in several of the following stages as well. As the number of cells increases the connecting films be-

come less easily recognized.

The second cleavage occurs about twenty-five or thirty minutes after the first. The plane of division is also meridional and at right angles to the first segmentation. It begins ~~next~~ at to the centre of the egg next to the furrow of the first cleavage and slowly extends out toward the periphery. When the division is completed the four blastomeres undergo a slight rotation from right to left; and in the centre of the egg between the cells there is, at times, to be seen a small open space or segmentation cavity which may extend through the entire egg as shown in Figure 12.

After a lapse of time equal to that which occurs between the first and second divisions, the third cleavage furrow appears. This plane of division is equatorial and divides the egg into eight blastomeres. When the segmentation is first completed the two quartets of cells are situated one upon the other and form a more or less spherical whole, as is the usual arrangement in eggs in which segmentation

is equal and regular. This arrangement of the blastomeres, however, is of very short duration, for soon a separation takes place between the cells of the lower quartet and two of them roll away from the plane of separation in one direction; the other two moving out in the opposite direction. In this migration the blastomeres move through an angle of 45 degrees or more, and finally come to lie in such a position as to form a semicircular plate as shown in Figures 13 and 14. The separation and rotation of the cells of one quartet seems to be constant in its occurence; but the final arrangement of the blastomeres is not always as regular and definite as that shown in the figures. At times they are more loosely and irregularly connected, and may assume relative positions similar to that shown by Metschnikoff for <u>Oceania armata</u> in Figure 34, Plate 1, of his "Embryologische Studien." In the case referred to the blastomeres are so spread out that the individuals, with three exceptions, touch only one of their fellows, thus

resembling a string of beads somewhat coiled.

With this separation and rolling apart, the regularity of arrangement of the cells in the segmenting egg is lost, and the stages from this point on become more and more irregular with each successive division up to the time when the re-adjustment takes place which is the beginning of the formation of the free-swimming embryo.

It is possible to distinguish, during these early cleavage stages, a layer of ectosarc around each individual blastomere. Later as the cells increase in number and become smaller, the ectosarc covering becomes less conspicuous and finally is lost from sight entirely.

After an interval of about one half an hour, the fourth segmentation begins. The divisions of the different cells no longer take place simultaneously; some occur a few minutes before others, but all are completed within a comparatively short time. So far as the cleavage itself is concerned, it is still equal and regular, but the arrangement of the blastomeres is no longer regular or definite. They apparently

follow no law of symmetry, and may come to lie in any position. Figures 15, 16 and 17 show three different forms which the cells of the sixteen cell stage acquire, and various other arrangements of the blastomeres were seen while studying the living eggs which could not be figured for want of space. However the three figures are sufficient to show that the general form of the egg in this stage may be very different. In Figure 15 it is possible to imagine a direct relationship to a preceding form just a little more irregular than is shown in Figure 14. In a form as represented in Figure 16 the descent of the different cells from the individual blastomeres of the eight cell stage is less easily recognized. Figure 17 shows an egg in which all sixteen blastomeres are spread out to form a flat plate one cell thick in the form of a quadrangle. One can easily conceive how this arrangement can have resulted from a regular eight cell stage in which the rotation of the cells of the one quartet was greater than that shown in Figure

13. The flat, spread out position of the cells at once suggests the idea that the egg may have been subjected to pressure. ~~And~~ This might have been the case if the eggs had been studied on a slide under a cover glass; but there is no evidence that pressure was the cause of this plate-like arrangement, for these forms were occasionally found among a variety of other forms while studying the living eggs in a small preparation dish in sea-water with a two-thirds objective. As the eggs present a number of different forms when subjected to the same external conditions, it seems that the cause of these differences must be sought in the nature of the egg itself rather than in any surrounding influences.

The later cleavages follow at intervals of about the same duration as in the preceding stages. The irregularities of arrangement of the blastomeres increase as the cells become more numerous. On account of the smallness of the blastomeres and the extreme opacity of the egg, it becomes impossible to follow the segmentation in detail any further.

Figures 18 - 21 show a few of the later stages of comparatively very regular forms. Figure 20 represents an egg in which the blastomeres are arranged in two main groups held together by a narrow isthmus of only one cell in thickness. Some eggs were separated into three or four thickened clusters that were joined together by small masses of connecting cells. In others there were smaller groups of blastomeres projecting out from the general mass of cells, thus giving the whole somewhat of an ameboid appearance. The term amoeba-like seems to most clearly represent the shape which some of these late segmentation stages assume, for if a simple outline of these remarkable and grotesque forms is drawn it has a general resemblance to an amoeba with thick blunt pseudopods. Whether these irregularities in the shape of the egg during late segmentation, and the tendency of the cells to arrange themselves into more or less distinct lobes is due to an amoeboid property of the cytoplasm of the egg, or to a tendency to multiply by division during cleavage, as was suggested by Metschnikoff for <u>Oceania</u> <u>ar</u>-

mata, there is not sufficient evidence to decide. It may be possible that both of these factors act in determining the shape of the segmenting mass of cells. And doubtless the membraneless character of the egg plays a part in these phenomena

PLANULA.

When segmentation is complete a solid embryo is formed which may at first be called a morula. Small spaces occur sometimes between the blastomeres during the different cleavage stages, but they are sooner or later obliterated by the crowding together of the cells. A central cleavage cavity which is later transformed into a blastocoele is not formed; consequently a true blastula does not exist in the development of Turritopsis. In this respect it differs very markedly from Stomotoca and the majority of hydro-medusae of which the development has been studied, in which a definite blastocoele is formed that becomes filled

finally with the migrating endoderm cells. When the developing egg is about six to eight hours old, the very irregular shape, which the segmenting mass has assumed, becomes less marked. Gradually the cells become rearranged; the lobes and processes which previously were so conspicuous are now drawn into the main mass of cells, and the egg is transformed into an oval embryo. This process of rounding up lasts from two to four hours. The cells of the embryo now develop cilia, and the larva begins to move. At first the movements are feeble, but soon the larva is able to leave the bottom of the aquarium and swim free in the water. Eggs that are laid at five to six o'clock in the morning develop to the free-swimming stage by four in the afternoon. The larva swims with its broad end forward! and has a spiral or cork-screw motion, which propels it onward. This method of swimming is common to hydroid larvae. When the embryo reaches this stage the cells become very numerous and small. And before the cilia are developed and

movement begins it resembles an unsegmented egg very much, except that instead of being spherical it is now oval. In size it is about the same as the unsegmented egg, if anything rather smaller. The decrease in size must be accounted for by the fact that some of the yolk has been digested; and the larva evidently has not yet acquired any means of receiving food from the external world.

The larva remains in this oval condition for some hours, after which it elongates to form a typical planula. When the embryo is twenty-four hours old it lengthens out and becomes more slender and assumes a general appearance as shown in Figure 23. As it becomes older it grows still longer. Figure 24 shows a larva of thirty hours. It has now the power of contraction; and is sensitive to stimuli. When the cilia are first developed and for some time during the oval condition of the larva it swims near the bottom of the aquarium. But as it grows longer and elongates it rises in the water and swims at or near the surface. The length

of time during which the embryo remains in the free-swimming planula stage is variable; but as a rule by the time it is about forty-eight hours old, it begins to sink toward the bottom of the aquarium, and to swim less rapidly. After the spiral swimming movements are lost, the planula is capable of gliding along the bottom of the dish for some time. Finally the motion ceases altogether and the larva loses its cilia and is ready for attachment. This stage of development is reached under favorable conditions about forty-eight to fifty hours after the eggs have been laid.

The planula is very opaque, and thus it is impossible to make out anything about its internal structure in studying the living forms. Specimens in various stages of development were preserved and sectioned for the study of cellular structure. The description of this structure will be given in connection with the formation of the germ layers.

Brooks describes and figures an ectodermal invagination

at the posterior end of the planula. He says: "In a living planula it is easy to make out the posterior end, an ectodermal invagination, which looks very much like the mouth of an invagination gastrula, but this resemblance is misleading, for the careful study of a similar structure in the planula of _Eutima_ shows that the invagination has no connection with the digestive cavity, but is an ectodermal gland for the attachment of the planula." From my observations I am forced to regard this structure, which he describes, as a variation rather than a normal feature. It seems to be an abnormal occurence which is found only rarely. Among the many specimens which I studied both in life and from preserved material, such an invagination was met with only on one occasion. Then it was at the anterior end of the planula instead of the posterior. These ~~features~~ structures are clearly abnormal features of the developing _Turritopsis_ planula.

EXPERIMENTAL.

The very irregular character of the segmenting egg and the loose connection of the blastomeres; and their tendency to separate into more or less definite lobes and protuberances, as has been described in the section on segmentation suggested the problem: What would be the effect of dividing the eggs during the comparatively early stages of cleavage? With this question in mind a few experiments were tried. The eggs were divided during several stages of segmentation. The best method for separating the cells was found to be by placing them on a clean glass plate moistened with sea-water. Then with a finely pointed needle or with a very delicate scalpel the blastomeres could be cut or torn apart without being crushed. After they were divided, they were flooded from the glass plate by water from a pipette into a dish of sea-water and watched in their development. The advantage of separating the eggs on a glass plate is that they are held slightly by surface tension, and do

not rotate as readily while being cut apart. Eggs were divided during different stages of cleavage from two to six hours old. They were then placed under conditions as nearly like those under which the eggs not divided developed as possible. Unfortunately, as these experiments were incidental and incomplete, no eggs were divided during the two-cell stage and their cleavage followed in detail. Some eggs that were laid between five and six in the morning were divided at 10.45 A. M. More than one half of the fragments continued to develop and by six o'clock in the evening had reached the free-swimming stage. They were retarded a little in their development; whole eggs usually arrive at this stage at about four to four-thirty. They were slightly smaller than embryos from whole eggs, but apparently just as active and normal, except in size. By the next morning they had reached the elongated planula stage and were in good condition, swimming at the surface of the water.

At another time some younger eggs were divided. These showed practically the same results in development. The opacity of these embryos made the study of their minute structure impossible during life; and because of scarcity of material none could be preserved to study their histology from sections. However these few incomplete experiments show that fragments of the egg of Turritopsis are capable of developing into apparently entire and normal embryos of slightly smaller size.

Hargitt artificially divided some Pennaria eggs during the first cleavage and figures a number of resulting segmentation stages, which ~~is~~ are very similar to those of whole eggs. He says: "As will be seen, each of the resulting halves behaved in a manner indistinguishable from that of normal eggs. These half embryos were followed through the entire process of cleavage and through the later metamorphoses into planula and polyp, and in every respect,

size alone excepted, the process was perfectly normal."

To my knowledge Haeckel was the first to publish the statement that halves of hydromedusa eggs would develop into normal embryos. For some time naturalists in general were inclined to doubt the fact; but since the work of Boveri, Hertwig brothers, Roux, Driesch, Wilson, Morgan, Loeb and others on the fragments of eggs, the development of embryos, abnormal and normal, from the portions of eggs is a question no longer to be doubted.

FORMATION OF THE ECTODERM.

In the development of the egg of Turritopsis the germinal layers are not differentiated by process of emibole, delamination or cellular ingression. During segmentation the blastomeres do not separate and arrange themselves around a segmentation cavity which later is transformed into a blastocoele. Thus instead of having formed a coeloblastula, we find that cleavage results in the formation of a solid

oval embryo destitute of a blastocoele, which may be called a morula stage. The cells of the segmenting egg are all alike in structure and nearly equal in size; so that they are not distinguishable into primitive ectoderm and primitive endoderm, which is the case in forms where a definite delamination takes place, as is so beautifully shown in Liriope and Geryonia, and in species where cellular ingression occurs as in Stomotoca and Clytia for example. Figures 25 to 30 illustrate the uniformity of the cells, and the solid character of the egg during segmentation. In Figure 27 a space exists between the blastomeres near one end of the egg, but this is not to be regarded as a true cleavage cavity. The next figure shows three of these false cleavage cavities. They occur only occasionally. As stated before most of the eggs are entirely solid.

About the time the irregular mass of segmenting blastomeres is metamorphosed into the oval embryo, the cell boundaries are lost for a short time and a syncytium is formed. This syncytial structure is crowded with yolk granules and

a number of nuclei are scattered through the protoplasm. The nuclei soon become more numerous near the periphery; and then cell walls begin to appear as shown in Figure 33. These cells are to become the ectoderm, which is soon separated from the inner structureless mass by the development of the mesogloea. Now the ectoderm forms a distinct layer, composed of columnar cells all of which are at first similar in structure and lie parallel to each other as shown in Figure 34. The differentiation of the ectoderm cells takes place later.

The formation of the germinal layers in _Turritopsis_ is different from that which has generally been described for the development of Hydromedusae. In the majority of forms previously studied the differentiation took place either by delamination or by cellular ingression, unipolar or multipolar. These methods have been well described and figured by Metschnikoff for a number of species.

In Aglaura and Rhopalonema there is found, according to Metschnikoff, a solid so-called morula stage destitute

of cleavage cavity, the superficial cells of which are converted into the ectodermal layer, while those within represent the endoderm. Here the two layers are formed directly without the formation of a syncytial structure.

In Eudendrium and Pennaria according to Hargitt's description a condition somewhat similar to that of Turritopsis is found. He says: "Indeed in both Eudendrium and Pennaria, not to mention other cases, cleavage would seem to result primarily in the formation of a more or less characteristic syncytium, the subsequent development of the germ layers taking place by a gradual differentiation of the syncytial elements, first and naturally the ectoderm, and later, often very much later, the endoderm."

The syncytial character in Turritopsis is acquired under favorable conditions, when the embryo is about six hours old; at the time that the irregular mass of segmenting cells is metamorphosed in to the oval embryo. And I am inclined to think that the formation of the syncytium

and the change of shape of the developing embryo are connected phenomena. The length of time during which this condition lasts is evidently comparatively short, for soon cilia develop and the larva begins to swim. ~~But~~ Meanwhile the peripheral region of the syncytium has been transformed into a distinct layer of ectodermal cells, separated from the inner mass of tissue, still structureless in character, by the development of the mesogloea.

From the fact that a syncytium, or plasmodium-like structure is formed, it is impossible to localize any of the blastomeres of the segmenting egg which will form special parts of the future embryo. Even those cells which are at the surface at the completion of segmentation cannot be regarded as primitive ectoderm, for in the breaking down of the cell boundaries, the formation of the syncytium, and the recasting of the cells it is quite impossible to say what change of the protoplasm may take place.

FORMATION OF THE ENDODERM.

The formation of the endoderm in Turritopsis cannot be adapted to any of the schemes of the development of the Hydromedusae which have been sketched by Metschnikoff. He distinguishes three principal methods for the development of the inner germ layer: First, delamination, a process in which the segmenting blastomeres divide in a plane nearly parallel to the surface; and the inner parts or cells become primitive endoderm, while the outer parts remain as primitive ectoderm. Second, multipolar ingression, in which cells migrate into the blastocoele from different regions of the peripheral cell layer, and are transferred into endodermal tissue directly. Of this mode he describes several subordinate types. Third, unipolar migration, similar to the preceding except that the primitive endoderm cells are given off at one pole only; at the posterior end of the larva.

In Turritopsis the endoderm is derived from the syn-

cytial mass of tissue left in the centre of the embryo after the ectoderm has been formed and separated off by the development of the mesogloea. The inner germ layer as a rule is formed much later than the ectoderm. Soon after the supporting membrane is developed cell boundaries begin to appear in the syncytium in the interior of the larva. The cells thus formed are primitive endodermal cells, and are crowded together without any definite arrangement for a number of hours. Stages in which the cell walls are reappearing are shown in Figures 34 to 36. When the embryo is about forty-eight to sixty hours old, the time at which attachment takes place, a fissure appears in the middle of the mass of endodermal tissue. This is the beginning of the coelenteric cavity. This separation begins near the anterior part and grows toward the posterior end. The coelenteron gradually increases in size, and at the same time the endodermal cells begin to be rearranged; and finally become situated parallel to each other with their bases

against the mesogloea and form a definite inner germ layer.

Gerd has observed in Bougainvillia that during the course of cell multiplication the cell boundaries become indistinct and that the peripheral and central nuclei are altogether identical. But this species differs from Turritopsis, according to his description in the formation of the compact morula stage, in that it is brought about by a multipolar migration of cells into the interior of the coeloblastula; while in Turritopsis the morula stage results directly from segmentation without any recognizable migration of cells.

The formation of the endoderm in Turritopsis therefore differs from nearly all the methods which have previously been described; and which in the main conform to one or another of the stereotyped methods as established by Metschnikoff. The nearest approach is that briefly described by Hargitt for Eudendrium and Pennaria, in which there is also more or less of a syncytium formed prior to the differenti-

ation of the germ layers.

CELL MULTIPLICATION.

During the early cleavage phases the cells multiply entirely by the process of mitosis. But in the later phases, especially when the egg is approaching that stage in which the cell boundaries are lost, there is good evidence that direct cell division is also of frequent occurence. In this period of development mitosis and amitosis take place simultaneously in the different cells of the segmenting egg. Figure 31a shows a karyokinetic spindle in the metaphase; and figure 31b one in the anaphase. The chromosomes are large and prominent; but are too closely crowded together to be counted with accuracy.

The nuclei which divide amitotically vary in size considerably, and have a reticular appearance. Figure 32a shows a large nucleus of this reticular character with the chromatin scattered about in the linin meshwork. Figures

78b to 78e illustrate nuclei in various stages of amitotic division. Frequently in cells where amitosis takes place many of the yolk granules have been digested and consequently are fewer than in cells where digestion is less active. It may be that the more active functions of digestion and the phenomena of direct cell division are associated with each other. Or it may be that the view of Flemming and Ziegler, that amitosis is connected with a high specialization of the cell or is the forerunner of degeneration, applies in this case. This latter conception seems plausable, for we find amitosis to be most abundant shortly before the cell boundaries disappear and the embryo is transformed into the syncytium.

For a number of years it has been known that amitosis is common in follicle cells, digestive epithelial cells, supporting cells, etc.; but generally it was not supposed to take place in early embryonic development. Within the last few years however a number of observers have discovered this

phenomenon in the developmental stages of various forms.

ATTACHMENT.

Under favorable conditions when the larva is about fifty hours old it reaches that stage of development at which attachment takes place. In preparation for this process the planula settles to the bottom, loses its cilia and consequently its movements cease. The manner of attachment in Turritopsis like that of Stomotoca differs from that usually described in hydroid development. Instead of settling down on the anterior end of the planula according to the method which occurs in Eudendrium, and which has been regarded as typical and used in descriptions of the embryology of the Hydromedusae in text-books, the planula becomes attached on its side by nearly its whole length, and is transformed into a root. The hydranth instead of growing up from the posterior end of the planula as in forms which attach themselves by the anterior end, de-

velops from a bud that is given off from the root, usually about the middle.

Professor Brooks observed the fact that the planula is transformed into a root in *Turritopsis*, *Eutima* and *Hydractinia*; and gives a brief account of the same in his paper on "The life-History of *Eutima*"(1884). Metschnikoff describes and figures for *Mitrocoma* the fact that the larva becomes attached by its side and is almost wholly employed in the formation of the hydrorhiza, while the first hydranth grows out of it by a kind of budding (Embryologische Studien, 1886).

In general the attachment of the planula is similar in *Turritopsis* to the method which is followed by *Stomotoca*, but the former does not commonly produce secondary hydrorhiza. In *Stomotoca* about the time the hydranth bud appears, or even before, the root branches giving rise usually to one or two secondary roots; In *Turritopsis* this branching rarely takes place, at least during the first few days of the de-

velopment of the hydranth.

Professor Brooks describes and figures in the planula of *Eutima* an ectodermal adhesive gland. It occurs after the endoderm and the digestive cavity are formed, and before the appearance of the mouth, as an ectodermal invagination at the small end of the planula. In *Turritopsis* no such special organ of attachment is found. The larva probably becomes fixed by a secretion extruded from the ectoderm cells along the whole length of its body.

DEVELOPMENT OF THE HYDRANTH.

Shortly after the larva becomes attached a bud develops, usually at about the centre of the root, which is the beginning of the first hydranth. Four small projections appear early around the distal part of the bud; these will later form the first circle of tentacles. At this time no mouth has yet developed. A young polyp in this stage of development is shown in Figure 37. The hydranth bud continues to grow taller and after a few hours a second whorl of ten-

tacular buds is formed some distance below the first circle of tentacles. When the polyp is from twenty to twenty-four hours old, or about seventy-two hours after the egg is laid, it is ready to develop the third whorl of tentacles. Thus the tentacles nearest the apex of the hydranth are the oldest and largest. The circles are indefinite, that is the tentacles of a whorl do not all arise from the same level; so that in the advanced hydroid they have rather the appearance of being scattered than arranged in circles. The tentacles when fully developed are stout and filiform; and are capable of much extension and contraction. Figures 37 to 41 illustrate various stages in the early development of the hydranth; the youngest being about fifty hours and the most matured some seventy hours old. Figure 39 shows a form in which the polyp arises from near the end of the hydrorhiza. This is exceptional. A hydranth with the third circle of tentacles is shown in Figure 41; the tentacles of the first whorl have become considerably elongated. The hydrocaulus now becomes longer and more slender; and the hydranth assumes a fusi-

form body.

The polyps that I reared from eggs at the age of three days were in the main features like the hydranths of the adult colony found and figured by Professor Brooks, except that they had not yet developed as many tentacles. In his description he says: "The upright stems of the hydra, from 8 mm. to 12 mm. high, bore large terminal hydranths, as well as smaller ones which were scattered irregularly along the stem on short stalks. The long fusiform body of the hydranth carries from eighteen to twenty thick, short, filiform tentacles, which are arranged in three or more indefinite whorls. The medusa buds originate around the stem just below the hydranths, and they are themselves carried on short stems. The perisarc is not annulated, and it forms a loose cylindrival sheath around the main stem, and the short branches which carry the lateral hydranths and the young medusae, while the latter are invested by a

much thinner and more transparent capsule of perisarc. The sheath of the stem is thick and crusted with foreign matter. It terminates abruptly by a sharp collar just below each hydranth. The young hydranths and the medusae are budded off above the collar, but they soon become entirely sheathed in perisarc by the growth of the stem. The pale yellowish-red hydranths are very similar to those of Tubularia (Allman) and the hydroid is so similar to Dendroclava Dohrnii recently described by Weismann, that they undoubtedly belong to the same genus."

SUMMARY.

1. The ova of Turritopsis arise in the ectoderm of the manubrium. They grow by the absorption of the primitive ovarian cells; and when mature are densely crowded with large yolk granules.

2. Dehiscence takes place at a definite time, from five to six o'clock in the morning.

3. The egg is spherical and membraneless. It is con-

posed of an outer layer of clearer ectoplasm and a central mass of endoplasm which is dense and opaque and filled with large, dark yolk spheres.

4. Maturation and fertilization take place in the water after the eggs are deposited. It is impossible to make out details in the living eggs because of their opacity.

5. Cleavage is total and nearly equal. The first three divisions are fairly regular; but during the later segmentation the arrangement of the blastomeres becomes very irregular and erratic. At the completion of segmentation a solid morula stage is formed, in which the cell boundaries are lost for a time giving rise to a syncytium.

6. Parts of eggs which are ~~divided~~ during the cleavage stages continue to develop and from larvae which are normal in every respect except size.

7. The ectoderm is formed by the reappearance of cell walls in the periphery of the syncytium mass; and is separated from the interior part by the formation of the mesogloea.

8. The formation of the endoderm follows none of the typical methods described by Metschnikoff. It arises late in the larval life from the syncytial mass of tissue left in the interior of the embryo after the separation of the ectoderm by mesogloea. When the cells first reappear they are crowded together without any definite arrangement; finally they come to form the distinct endodermal layer.

9. During the late segmentation there is evidence that some of the nuclei divide amitotically.

10. The planula becomes attached on the side by nearly its entire length, and is transformed into a root.

11. The first hydranth develops from a bud which is given off at about the middle of the root soon after attachment.

12. The tentacles develop in indefinite whorls. Each whorl has four tentacles. The oldest are nearest the distal end. In the fully developed hydranth they have the appearance of being scattered rather than being arranged in circles.

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